PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

4/4

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/US2004/031230	International filing date *(day/month/year)* 23.09.2004
Priority date *(day/month/year)* 24.09.2003	
International Patent Classification (IPC) or both national classification and IPC G01N33/68, C12N15/81, C12Q1/02	
Applicant UNIVERSITY OF CHICAGO	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

Pellegrini, P

Telephone No. +49 89 2399-5729

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/031230

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	3,4,6-24
	No:	Claims	1,2,5
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-24
Industrial applicability (IA)	Yes:	Claims	1-24
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents:

D1: EVIN GENEVIEVE ET AL: "Presenilin I expression in yeast lowers secretion of the amyloid precursor protein" NEUROREPORT, vol. 11, no. 2, 7 February 2000, pages 405-408.

D2: EDBAUER D ET AL: "RECONSTITUTION OF GAMMA-SECRETASE ACTIVITY" NATURE CELL BIOLOGY, MACMILLAN PUBLISHERS, GB, vol. 5, no. 5, May 2003, pages 486-488, cited in the application.

D3: WO 01/16355 A (BOEHRINGER INGELHEIM PHARMA ; FUCHS KLAUS (DE); KOSTKA MARCUS (DE); FE) 8 March 2001.

D4: WO 98/15828 A (SCIOS INC ; CORDELL BARBARA (US); HIGAKI JEFFREY N (US)) 16 April 1998.

2. The subject-matter of claims 1, 2 and 5 is not novel (article 33(2) PCT):

D1 (abstract; page 405, right-hand column, para.2 - page 406, left column, para.2) discloses *Pichia pastoris* transfected with presenilin 1 and APP.

2.1. The subject-matter of dependent claims 3, 4 and 6-18 as well as of independent claim 19 is novel, as the technical features of these claims are not disclosed by the cited prior art.

3. The subject-matter of claims 20-24 is novel (article 33(2) PCT).
The cited prior art does not disclose a method for identifying gamma-secretase inhibitors, comprising the use of *Pichia pastoris* transfected with presenilin 1, APH-1, nicastrin and PEN-2.

4. The subject-matter of independent claim 20 is not inventive (article 33(3) PCT).

a. D3 (claims 1, 6 and 7), representing the closest prior art, discloses a method for identifying gamma-secretase inhibitors, comprising using cellular extracts from H4 cells expressing endogenous gamma-secretase activity in presence of APP or APP C99.

b. The main difference between claim 20 and D3 is that a yeast cell transfected with presenilin 1, nicastrin, APH-1 and PEN-2, and therefore expressing exogenous gamma-secretase activity, is employed. The technical effect of this difference is a simplification of the method of D3, as manipulating yeast cells is easier and less demanding than manipulating mammalian cells. The technical problem of claim 20 in view of the closest prior art is therefore to provide an improved method of identifying gamma-secretase inhibitors. The solution proposed is a method involving the use of the yeast *P.pastoris* transfected with presenilin 1, nicastrin, APH-1 and PEN-2, and therefore expressing exogenous gamma-secretase activity. Such solution is obvious to the skilled person, as D2 discloses the yeast *Saccharomyces cerevisiae* transfected with presenilin 1, nicastrin, APH-1 and PEN-2, and expressing exogenous gamma-secretase activity. It would be obvious for the skilled person to combine the teachings of D3 and D2 to arrive at a method of screening involving the yeast *S.cerevisiae* transfected with presenilin 1, nicastrin, APH-1 and PEN-2, considering the advantages of using yeast cells rather than mammalian cells (see above). Furthermore, *P.pastoris* is a well-known alternative to *S.cerevisiae* as eucaryotic cell for expressing recombinant proteins; the use of *P.pastoris* transfected with presenilin and APP in evaluating secretase activity is disclosed by D1 (page 406, right-hand column, para.4 - page 408, left column, para.1); and the use of *P.pastoris* rather than *S.cerevisiae* is not associated with any demonstrated technical effect. For these reasons, a method of screening based on *P.pastoris* is to be considered as an obvious alternative to a method of screening based on *S.cerevisiae*.

4.1. Dependent claim 21 and 22 are also not inventive, as the use of solubilized membrane preparation is conventional in the technical field considered and the use of APP and of APP C99 is disclosed by D3.

5. The subject-matter of claims 23 and 24 is also not inventive for analogous reasons as discussed above in point 4 and considering D4 rather than D3 as the closest prior art. D4 (claim 1) discloses a method for identifying gamma-secretase inhibitors, comprising using cells expressing endogenous gamma-secretase activity in presence of APP.

6. The subject-matter of claims 3, 4 and 6-19 is not inventive, as these claims refer to the *P.pastoris* expression system used to perform the non-inventive methods of claims 20-24. The APP mutations defined by claims 15 and 17 are well known, as discussed in the present description (pages 1-3).